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Impaired exercise capacity in oost-COVID syndrome: the role of VWF-ADAMTS13 axis

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Abstract:
Post-COVID syndrome (PCS) or Long-COVID is an increasingly recognised complication of acute SARS-CoV-2 infection, characterised by persistent fatigue, reduced exercise tolerance chest pain, shortness of breath and cognitive slowing. Acute COVID-19 is strongly linked with increased risk of thrombosis; a prothrombotic state, quantified by elevated Von Willebrand Factor (VWF) Antigen (Ag):ADAMTS13 ratio, and is associated with severity of acute COVID-19 infection. We investigated if patients with PCS also had evidence of a pro-thrombotic state associating with symptom severity. In a large cohort of patients referred to a dedicated post-COVID-19 clinic, thrombotic risk including VWF(Ag):ADAMTS13 ratio, was investigated. An elevated VWF(Ag):ADAMTS13 ratio ({greater than or equal to}1.5) was raised in nearly one-third of the cohort and four times more likely in patients with impaired exercise capacity as evidenced by desaturation {greater than or equal to}3% and/or rise in lactate level more than 1 from baseline on 1-minute sit to stand test and/or 6-minute walk test ($p<0.0001$). 20% (56/276) had impaired exercise capacity, of which 55% (31/56) had a raised VWF(Ag):ADAMTS13 ratio {greater than or equal to}1.5 ($p<0.0001$). FVIII and VWF(Ag) were elevated in 26% and 18% respectively and support a hypercoagulable state in some patients with PCS. These findings suggest possible ongoing microvascular/endothelial dysfunction in the pathogenesis of PCS and highlight a potential role for antithrombotic therapy in the management of these patients.

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Key Points:

1. VWF(Ag):ADAMTS13 ratio ≥1.5 was evident in 28% of PCS cohort.
2. 55% of patients with impaired exercise capacity had a raised VWF(Ag):ADAMTS13 ratio ≥1.5 (OR 4)

Abstract

Post-COVID syndrome (PCS) or Long-COVID is an increasingly recognised complication of acute SARS-CoV-2 infection, characterised by persistent fatigue, reduced exercise tolerance chest pain, shortness of breath and cognitive slowing. Acute COVID-19 is strongly linked with increased risk of thrombosis; a prothrombotic state, quantified by elevated Von Willebrand Factor (VWF) Antigen (Ag):ADAMTS13 ratio, and is associated with severity of acute COVID-19 infection. We investigated if patients with PCS also had evidence of a pro-thrombotic state associating with symptom severity. In a large cohort of patients referred to a dedicated post-COVID-19 clinic, thrombotic risk including VWF(Ag):ADAMTS13 ratio, was investigated. An elevated VWF(Ag):ADAMTS13 ratio (≥1.5) was raised in nearly one-third of the cohort and four times more likely in patients with impaired exercise capacity as evidenced by desaturation ≥3% and/or rise in lactate level more than 1 from baseline on 1-minute sit to stand test and/or 6-minute walk test ($p<0.0001$). 20% (56/276) had impaired exercise capacity, of which 55% (31/56) had a raised VWF(Ag):ADAMTS13 ratio ≥1.5 ($p<0.0001$). FVIII and VWF(Ag) were elevated in 26% and 18% respectively and support a hypercoagulable state in some patients with PCS. These findings suggest possible ongoing microvascular/endothelial dysfunction in the pathogenesis of PCS and highlight a potential role for antithrombotic therapy in the management of these patients.

Introduction

The emergence of the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has led to significant morbidity and mortality on a global level with 257,662,742 confirmed cases of COVID-19 and 5,166,671 deaths[1] at the time of writing. Significant advances have been made in the clinical management of severe COVID-19 disease in the hospitalised population[2]. However, just under 10% of patients with COVID-19 are admitted to hospital,

the majority of people infected with COVID-19 are not hospitalised[3]. A proportion of cases experience ongoing symptoms, dominated by fatigue, chest pain, shortness of breath and cognitive slowing. This has been named Long-COVID by patient groups, and termed post-COVID syndrome (PCS) by the National Institute for Health and Care Excellence (NICE)[4]. Estimates from the Office for National Statistics suggest that 1.1 million people in the UK are suffering from PCS[5]. The NICE guidance on the long term effects of COVID-19 provide both definitions for illness and a framework for clinical management in PCS clinics, recently endorsed by the WHO[6]. It has been defined as a condition that "occurs in individuals with a history of probable or confirmed SARS CoV-2 infection, usually 3 months from the onset of COVID-19 with symptoms and that last for at least 2 months and cannot be explained by an alternative diagnosis"[4].

Acute SARS-CoV-2 infection generates a hypercoagulable state, particularly evident in hospitalised patients[7]. Raised D-dimer levels which has been associated with poor outcomes[7], as well as elevated factor VIII (FVIII) and Von Willebrand factor (VWF) levels further support the hypercoagulability hypothesis[8]. VWF plays a major role in primary haemostasis and multimeric size is regulated by ADAMTS13 (A Disintegrin and Metalloprotease with Thrombospondin 1 repeats, number 13). In acute COVID-19 infected patients, a rise in VWF:Ag (antigen) which may be associated with a relative reduction in ADAMTS13 and therefore an increase in VWF(Ag):ADAMTS13 ratio is recognised and this is likely to contribute to the hypercoagulable state and risk of microvascular thrombosis in these patients.[8,9] Enhanced thrombin generation, decreased fibrinolytic activity, raised levels of FVIII and plasminogen-activator inhibitor type 1 (PAI-1),[10,11] high endothelin-1 levels,[12] and raised D-dimer[13] has also been presented in PCS patients. These suggest a role for endothelial cell dysfunction and hypercoagulability.

We hypothesised that the pro-thrombotic state is implicated in the pathogenesis of PCS. We investigated coagulation parameters associated with reduced exercise capacity in patients with PCS, identifying the utility of these parameters in PCS to determine ongoing disease activity. We further investigated if an association exists between elevated VWF(Ag):ADAMTS13 ratio and impaired exercise capacity in patients with PCS at a dedicated clinical service for PCS.

Methods

Of the 1471 face to face appointments seen in a dedicated outpatient post-COVID clinic between July 2020 to May 2021, baseline demographics were assessed retrospectively in

patients who had VWF(Ag):ADAMTS13 ratio tested. Of the 413 patient episodes, 352 were more than 3 months after acute infection with COVID-19. 22 episodes were repeat measurements performed over time and excluded from analysis unless specified, leaving 330 patients for full analysis. Trends in patients with repeat measurements are shown separately. Patients followed up in the clinic had proven or presumed (when PCR testing was not widely available during initial stages of pandemic) COVID-19 infection. A clinical syndrome compatible with COVID-19 infection was agreed by two independent senior clinicians in those with presumed COVID-19 infection. Referral to the post COVID clinic included both patients post hospitalisation and referrals from community non-hospitalised cases. Patients attending the clinic did not have blood tests performed if they were completed by their general practitioner as part of the referral to the post-COVID clinic, or if recent blood test results were available through attendance to the hospital emergency or other departments. Blood tests were not repeated in patients reporting symptom improvement and this decision was not influenced by any other baseline characteristics such as age.

ADAMTS13 activity was measured using FRETS VWF73 assay[14]. Von Willebrand screen (VWF antigen and VWF activity) was measured using standard automated immunoturbimetric assay in a Sysmex CS-2500 analyser with a Siemens kit (VWF:Ag and INNOVANCE VWF activity, Siemens Healthcare Diagnostics, Marburg, Germany), and a clotting based assay using Factor VIII (FVIII) deficient plasma (reference OTXW17) and Dade Actin FS Activated PTT reagent (reference B4218-100) was used to measure FVIII. All analysis was performed on plasma derived from peripheral blood samples collected in sodium citrate tubes, as part of routine assessment in the post COVID clinic which included D-dimer.

VWF(Ag):ADAMTS13 ratio was calculated on 50 voluntary normal controls (Medical Research Ethics Committee Numbers 08/H0810/54 and 08/H0716/72). The VWF(Ag):ADAMTS13 ratio was also calculated in the PCS patient cohort and correlated with symptoms including exercise tolerance as assessed by 1 minute sit-to-stand test and/or 6 minute walk test. The 6 minute walk test (6MWT) and 1 minute sit-to-stand (STS) test were performed to assess abnormalities in components of exercise testing and therefore as an indicator of impaired exercise capacity. The 1 minute STS test involves going from sit to stand as many times as possible in 1 minute. Desaturation and number of repetitions completed as part of the STS test were examined. The 6MWT, as outlined in the American Thoracic Society (ATS) guidelines[15], is a simple practical test which requires a 100 feet hallway but without the requirement for complex or additional equipment. It measures the

distance that a patient can quickly walk on a flat surface in 6 minutes. Partial pressure of oxygen, pH and capillary lactate were measured from an ear-prick blood test pre and post exercise during the 6MWT, in parallel with continuous peripheral oxygen saturation measurement. Peripheral oxygen desaturation ≥3% for 6MWT and STS test, as well as an increase in lactate >1 from baseline during 6MWT were taken as markers of impaired exercise capacity. Reference values for the 6MWT has been assessed in healthy subjects aged 20-50 years and the mean oxygen saturation after walking was 97±1.3% with a mean change in saturation of 1±1.1%[16].

293/330 patients had STS testing performed and these were analysed in more detail with focus on the number of repetitions completed. 1 minute STS testing in COPD patients has previously shown 19±6 repetitions on average[17] and similarly 21±6 in interstitial lung disease[18]. This test has also been used to evaluate physical capacity and exertional desaturation one month post discharge in patients who survived COVID-19 pneumonia. The average number of repetitions was 20.9±4.8 in these patients[19]. Repetitions ≤20 was therefore considered as an indicator of fatigue. Correlation between these components of the sit-to-stand test and VWF(Ag):ADAMTS13 ratio was assessed. Potential associations with D-dimer results were also investigated.

Statistics

All statistical analysis was performed using Graphpad Prism 9. Continuous data was summarised as median and interquartile range (IQR) with use of the Mann Whitney test to compare ranks across 2 groups, and Kruskal-Wallis testing was used to compare ranks across 3 groups. Gaussian distribution was not assumed for the comparison between groups in this paper, as variables showed signs on non-normality, in particular a long right tail for VWF(Ag):ADAMTS13 ratio (Figure 1a). VWF(Ag):ADAMTS13 ratio was calculated by (VWF(Ag)/ADAMTS13 activity) X 100 in each set of results for patients and normal controls. Number and percentage was used to summarise categorical data. Chi squared/Fishers exact test was used to assess statistical significance in categorical variables. Odds ratios (OR) and 95% confidence intervals (CI) were estimated using logistic regression models. A p-value<0.05 was considered statistically significant.

Results

The baseline demographics of 330 patients were analysed. 60% were female and 40% were male, with a median age of 46 years (range 18-88 years). Patients followed up in clinic varied from 3 to 15 months post proven or presumed COVID-19 infection. 320/330 (97%)

had symptoms greater than 3 months following acute COVID-19 infection, of which respiratory symptoms and fatigue were commonest (Table 1).

85/330 (26%) of PCS patients had high FVIII ranging from 2.1 to 5.1IU/ml (normal range (NR) 0.5-2IU/ml) and 60/330 (18%) had high VWF(Ag) ranging from 1.7-3.3IUml (NR 0.5-1.6 IU/ml). ADAMTS13 activity ranged from 68.2 to 159.3IU/dL (NR 60-146IU/dL).

The median VWF(Ag):ADAMTS13 ratio was 0.98 with an interquartile range of 0.76-1.34 in 50 historical stored normal control samples. Based on this interquartile range, a VWF(Ag):ADAMTS13 ratio of 1.5 or greater was considered raised. The median VWF(Ag):ADAMTS13 ratio was 1.2 (IQR 0.9-1.5) across the 330 patients. 28% (92/330) of patients had a VWF(Ag):ADAMTS13 ratio ≥1.5 across the studied cohort.

D-dimer was raised (>550ug/l FEU) in 42/330 (13%) tested. Elevated D-dimer was associated with a median VWF(Ag):ADAMTS13 ratio of 1.3 (IQR 1.0-1.6) compared to a ratio of 1.1 (IQR 0.9-1.5) in the 240/330 (73%) that had a normal D-dimer (p=0.08). 48 patients did not have D-dimer testing.

VWF(Ag):ADAMTS13 ratio was divided into two groups: <1.5 (normal) and ≥1.5 (abnormal) and assessed separately against patient symptoms and results of exercise tolerance testing via the 1 minute sit-to-stand exercise test and/or 6 minute walk test. Of the 97% of symptomatic patients, 72% (230/320) had VWF(Ag):ADAMTS13 ratio <1.5 and 28% (90/320) had a ratio ≥1.5. 3% of the total cohort (10/330) was asymptomatic and had a median VWF(Ag):ADAMTS13 ratio of 1.1. Therefore, VWF(Ag):ADAMTS13 ratio did not correlate with presenting clinical symptoms

However, VWF(Ag):ADAMTS13 ratio≥1.5 did correlate with abnormal exercise intolerance. Of the 330 patients assessed, 54 (16%) did not undergo exercise testing or failed to complete the test. 276 (84%) completed exercise testing (Figure 1b). 220/276 (80%) were able to perform these tasks without impairment. Within this group, 171/220 (78%) had a normal VWF(Ag):ADAMTS13 ratio <1.5 and 49/220 (22%) had a VWF(Ag):ADAMTS13 ratio>1.5. 56/276 (20%) had evidence of abnormal exercise testing as confirmed by desaturation ≥3% and/or an increase in lactate levels >1 above baseline. Of these, 31/56 (55%) had a high ratio ≥1.5 (Table 2). This was equivalent to 11% of the whole cohort. Abnormal exercise testing was associated with a higher VWF(Ag):ADAMTS13 ratio with a median of 1.5 (IQR 1.2-1.7) compared with 1.1 (IQR 0.9-1.4) in those with normal exercise test (p<0.0001, figure 1c). The risk of a raised VWF(Ag):ADAMTS13 ratio was found to be 4

times more likely in those with an abnormal exercise test (OR 4.3 [95% CI 2.3-7.9], p<0.0001), demonstrating a strong association by univariate analysis. The OR was maintained (4 [95% CI 2.1-7.8]) by multivariate analysis after adjustment for age, gender and other co-morbidities as potential confounders.

Analysis of the 1 minute sit-to-stand test was performed focussing particularly on the number of repetitions completed. Repetitions ≤20 was considered indicative of fatigue. Of the 293 STS tests analysed, 228 were more than 3 months since acute COVID-19 infection and were used for analysis. 12 patients did not have repetitions recorded, leaving full analysis to be completed in 216 patients (no repeat measurements were included). 156/216 patients completed more than 20 repetitions and of these only 35/156 (22%) had a VWF(Ag):ADAMTS13 ratio ≥1.5. Conversely, 60 patients completed ≤20 repetitions and 19/60 (32%) had a VWF(Ag):ADAMTS13 ratio ≥1.5. However, no significant difference was found between these groups (p=0.17).

VWF(Ag):ADAMTS13 ratio and significant desaturation (≥3%) was analysed in relation to number of repetitions completed in the STS test (Table 3). 15% (9/60) of those with ≤20 repetitions had a significant drop in oxygen saturation ≥3%, of which 67% (6/9) had a VWF(Ag):ADAMTS13 ratio ≥1.5. The median ratio was 1.6 (IQR 1-1.8) compared to a normal ratio of 1.2 (IQR 0.9-1.5) in the 85% (51/60) who did not have a significant drop in saturations (p=0.07)). Comparably, in those who completed more than 20 repetitions, 13% (21/156) had a drop in oxygen saturations ≥3% with a median ratio of 1.5 (IQR 1.2-1.9) compared to 1.1 (IQR 0.8 -1.4) in the 87% (135/156) with no significant desaturation (p<0.0001). Overall 63% (19/30) of those who desaturated ≥3% had a raised VWF(Ag):ADAMTS13 ratio (p<0.0001). Desaturation ≥3% on a STS testing is associated with a high VWF(Ag):ADAMTS13 ratio.

The split of abnormalities in STS and 6MWT were assessed. Desaturation ≥3% was the most frequent abnormality in 29/56 (52%) patients, followed by lactate rise in 16/56 (29%) and desaturation plus lactate rise in 11/56 (20%). Median VWF(Ag):ADAMTS13 ratios were 1.5 (IQR 1-1.7), 1.3 (IQR 0.9-1.5) and 1.6 (IQR 1.6-1.9) respectively. 9/11 (82%) of those with desaturation >3% and a rise in lactate had a VWF(Ag):ADAMTS13 ratio≥ 1.5 compared to 17/29 (59%) in those that desaturated and 6/16 (38%) in those who had a rise in lactate alone (p=0.07). Elevated D-dimer levels (>550ug/l FEU) did not associate with desaturation or lactate rise (p=0.50).

VWF(Ag):ADAMTS13 ratio and exercise test results were analysed according to 3 age groups: ≤40 years, 41-60 years and >60 years. The median VWF(Ag):ADAMTS13 ratio was found to be 1 (IQR 0.8-1.3), 1.2 (IQR 1-1.5) and 1.6 (IQR 1.2-2) respectively (p<0.0001), showing a difference in ratio across these age groups. Exercise testing within these age groups showed whilst fewer patients had evidence of abnormal compared to normal exercise testing (abnormal exercise test in 16% (14/86) of patients <40 years, 17% (26/150) of patients 40-60 years and 41% (16/39) in patients >60 years), this was seen in a greater proportion of patients over 60 years suggesting an age effect (p=0.003).

Pre-existing lung co-morbidities were also analysed in relation to VWF(Ag):ADAMTS13 ratio and exercise test results. 45/330 (14%) had a pre-existing diagnosis of lung disease. No difference in median VWF(Ag):ADAMTS13 ratio was found in those with and without pre-existing lung disease – 1.1.(IQR 0.9-1.7) and 1.2 (IQR 0.9-1.5) respectively (p=0.80). Of the 56 patients with abnormal exercise testing, only 7 (13%) with an abnormal exercise test had pre-existing lung disease compared to 49/56 (88%) who did not. In the 7 patients with known lung disease - 4 had asthma (all with good control except 1 patient), 2 had chronic obstructive pulmonary disease (both were on treatment) and 1 had recent respiratory tuberculosis (TB) infection. Of the 220 patients with normal exercise testing, 28/220 (13%) had known lung disease compared to 192/220 (87%) who did not (p>0.99). Therefore, no significance effect of pre-existing lung disease was associated with an abnormal exercise test.

Median FVIII and VWF antigen levels were higher in patients with abnormal exercise tolerance (Table 4). No significant difference in D-dimer levels above 550ug/l FEU was found between those with normal and abnormal exercise tolerance (780ug/l FEU and 780ug/l FEU respectively, p=0.5).

273/330 patients had acute COVID-19 infection managed in the community. Community-managed (83%) and hospitalised patients (16%) had a comparable median VWF(Ag):ADAMTS13 ratio of 1.2 (IQR 1-1.4 and 0.9-1.5 respectively (p=0.71)). No significant difference in median VWF(Ag):ADAMTS13 ratio was seen in the hospitalised patients that required oxygen support, non-invasive ventilation or intubation and ventilation, with median follow up ratios of 1.2 (IQR 1-1.4), 1.1 (IQR 0.7-1.4) and 1.3 (1.2-1.6) respectively (0.22). 21/53 hospitalised patients received dexamethasone. The median VWF(Ag):ADAMTS13 ratios at follow up were 1.3 and 1.1 in patients who received dexamethasone and those who did not (p=0.10).

Finally, changes in VWF(Ag):ADAMTS13 ratio and exercise test results in 18 patients who had repeat assessments performed over time were analysed (Table 5). Findings were variable from a stable VWF(Ag):ADAMTS13 ratio to a reduction in ratio. Exercise testing was not repeated in all patients.

Discussion

The longer term impact of COVID-19 infection is now recognised to be associated with ongoing symptomatology beyond 3 months following acute infection; 22.1% of patients experience at least one symptom at five weeks following COVID-19 infection and 9.8% at twelve weeks[20] and high symptom burden post infection in non-hospitalised as well as post-hospitalised patients have been shown[21]. A thrombo-inflammatory basis to the pathogenesis of COVID-19 infection has been implicated. A raised FVIII, VWF and D-dimer are the primary haemostasis factors supportive of a hypercoagulable state. Analysis of these coagulation parameters led to investigation of VWF(Ag):ADAMTS 13 ratio and a raised ratio was particularly seen in the most severe COVID-19 cases[8,9]. We extended analysis of the VWF(Ag):ADAMTS13 ratio to patients with PCS and identified nearly a third of the cohort had an increased ratio.

The pathophysiology of PCS has not been adequately addressed. This is the first report to our knowledge to identify and report an association between a raised VWF(Ag):ADAMTS13 ratio and impaired exercise capacity, specifically related to desaturation $\geq$3% and a rise in lactate levels more than 1 above baseline. We found an association between a raised VWF(Ag):ADAMTS13 ratio and limited exercise capacity, in both hospital and non-hospital cases. A raised ratio was found to be four times more likely in patients with an abnormal exercise test. These findings suggest an ongoing prothrombotic state in PCS, demonstrated by measurable laboratory and clinical parameters, therefore adding confirmation to the hypothesis of microvascular/endothelial dysfunction in the pathogenesis of PCS. We hypothesise the persistence of a hypercoagulable state which may be associated with endothelial dysfunction and microthrombi in the capillary bed of large muscles, causing a reduction in oxygen capacity during exercise, resulting in anaerobic respiration and fatigue.

A markedly high VWF(Ag):ADAMTS13 ratio with a median of 6.07 has been shown during the acute phase COVID-19 infection[8]. Measurement of VWF(Ag):ADAMTS13 levels was not increased when analysed against a number of clinical symptoms, including fatigue, headaches and abnormalities in cognition, with a median VWF(Ag):ADAMTS13 ratio of 1.2 in our PCS cohort who had a median of 6 months following acute COVID-19 infection. This therefore suggests that the high VWF(Ag):ADAMTS13 ratio observed in patients with acute

COVID-19 infection may settle over time despite ongoing symptoms of PCS, with a reversal of the ratio to normal in the majority of patients. However, a limitation is that longitudinal measurements repeated over time are lacking in this study. The VWF(Ag):ADAMTS13 ratio remained greater than 1.5 in a proportion of patients with impaired exercise capacity measured objectively.

Virus dependent and independent mechanisms have been implicated in the pathogenesis of PCS, particularly in patients with respiratory symptoms. Evidence of myocardial inflammation in patients experiencing chest pain has been shown on magnetic resonance imaging (MRI). ACE2 downregulation, inflammation and immunological responses affecting the structural integrity of the heart have been suggested as potential mechanisms of PCS. A role for direct viral invasion, as has been found in heart tissue of patients with COVID-19 at autopsy has also been implicated[22]. A physiological basis to PCS, with measurable patient reported outcomes and organ impairment shown through biochemical and imaging characterisation of organ function with quantitative MRI has been shown in the ongoing COVERSCAN study[23].

Whole blood viscoelastic and thrombin generation tests support a hypercoagulable state in acute COVID-19 infection. Raised FVIII levels and plasminogen-activator inhibitor type 1 (PAI-1), and a fall in the previously higher admission levels of plasmin-antiplasmin (PAP) complexes, at 4 month follow up post COVID-19 infection suggest a hypercoagulable and hypofibrinolytic, and therefore prothrombotic state in PCS[10]. Pretorius et al[24] have shown the presence of large amyloid fibrin deposits in plasma of patients with acute COVID-19 infection. Importantly, these deposits were found to persist in patients with PCS and were resistant to fibrinolysis. A significant increase particularly in Serum Amyloid A and α(2)-antiplasmin that were trapped in the fibrinolytic resistant pellet was also found.
Mandal et al[25] also reported persistent raised D-dimer levels in 30.1% of patients post discharge for COVID-19 infection with symptom predominance for breathlessness and fatigue, and Townsend et al[13] showed that D-dimer levels remained increased in 25.3% of patients up to 4 months post acute COVID-19 infection. Raised D-dimer was found in only 13% of our PCS cohort who were more than 3 months following acute COVID-19 infection. Longer duration following COVID-19 infection in our cohort may explain this difference. Fogarty et al[26] demonstrated a role for sustained endotheliopathy in PCS, supported by increased endogenous thrombin potential and peak thrombin levels, as well as raised VWF antigen, VWF propeptide and FVIII. They showed a significant inverse correlation between 6MWT distances and both VWF antigen and VWF propeptide, which was however lost after adjustment for age, sex and severity of initial infection. Our results provide further support to the role of endothelial dysfunction in the pathogenesis of PCS. However, in addition to this,

we have provided evidence of an association between a raised VWF(Ag):ADAMTS13 ratio≥1.5 and impaired exercise capacity as evidenced by desaturation≥3% and/or rise in lactate levels. Desaturation ≥3% on a 1 minute STS test was specifically associated with a high VWF(Ag):ADAMTS13 ratio

These findings support screening patients referred with PCS for VWF(Ag):ADAMTS13 ratio and exercise testing. In a time where the pathogenesis of this newly arisen phenomenon is not yet clear, it is important from a research perspective to do these screening tests. The underlying mechanism that leads to abnormalities of components of exercise testing in this group of patients with a VWF(Ag):ADAMTS13 ratio ≥1.5 remain unanswered. Importantly, it also suggests a potential role for antithrombotic therapy in this cohort of patients and requires large scale trials to evaluate this as a management option in PCS sufferers.

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Author Contributions

N.P. analyzed data and wrote manuscript.
M.H., T.H., E.W., R.B. designed research, collected data and reviewed manuscript.
A.K., A.D. and A.D. collected data and reviewed manuscript.
L.N. and D.S. undertook laboratory testing and reviewed manuscript.
HM.D. oversaw statistical analysis and reviewed manuscript.
M.S. was senior author, designed research and reviewed manuscript.

Conflicts of Interest

M.S. received speaker fees and on advisory boards for Alexion, Novartis, Takeda, Sanofi and Octapharma; received research grants from Shire and Alexion.
The remaining authors declare no competing financial interests.

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Figure 1a. Histogram showing distribution of the VWF(Ag):ADAMTS13 ratio across the post-COVID patient cohort.

Figure 1b. Flowchart showing performance in exercise testing and associated VWF(Ag):ADAMTS13 ratio.

Figure 1c. Median VWF(Ag):ADAMTS13 ratio in patients with normal and abnormal exercise tests. Median VWF(Ag):ADAMTS13 ratio was 1.5 in patients with an abnormal exercise test compared to median ratio of 1.1 in patients with a normal exercise test ($p<0.0001$).

Table 1. Baseline demographics and proportion of patients reporting symptoms at time of clinic visit.

Total number of patients analysed (>3 months since acute COVID-19 infection)	330
Female:Male	60% (197/330):40% (133/330)
Median age	46 years (range 18-88 years)
Median time (months) from acute COVID-19 infection	6 (4-10)
Symptomatic patients	319/330 (97%)
Respiratory symptoms (breathlessness, cough)	223/330 (68%)
Fatigue	239/330 (72%)
Neurological symptoms (headaches, numbness, cognitive impairment (poor memory/concentration), postural symptoms, disturbed sleep, dizziness/light headedness)	153/330 (46%)
Cardiac symptoms (chest discomfort, palpitations)	155/330 (47%)
Rheumatological symptoms (arthralgia, myalgia)	79/330 (24%)
Abdominal symptoms (abdominal pain, nausea/vomiting, diarrhoea/constipation)	39/330 (12%)
Other (rash, anosmia, sore throat, splinter haemorrhages)	45/330 (14%)

Table 2. Proportion of patients with normal and raised VWF(Ag):ADAMTS13 ratios split according to exercise test result. Significantly more patients with an abnormal exercise test had a VWF(Ag):ADAMTS13 ratio≥1.5 (p<0.0001, OR 4).

	Normal exercise test	Abnormal exercise test
VWF(Ag):ADAMTS13 ratio<1.5	170/219 (78%)	25/56 (45%)
VWF(Ag):ADAMTS13 ratio≥1.5	49/219 (22%)	31/56 (55%)

Table 3. Proportion of patients with evidence of desaturation, median ratios in these groups and % with VWF(Ag):ADAMTS13 ratio≥1.5.

	Repetitions≤20 (n=60)		**Repetitions>20** (n= 156)	
	Desaturation≥3% 9/60 (15%)	**Desaturation<3%** 51/60 (85%)	**Desaturation≥3%** 21/156 (13%)	**Desaturation<3%** 135/156 (87%)
Median ratio (IQR)	1.6 (1-1.8)	1.2 (0.9-1.5) P=0.07	1.5 (1.2-1.9)	1.1 (0.8-1.4) P<0.0001
% with ratio≥1.5	6/9 (67%)	13/51 (25%) P=0.02	13/21 (62%)	22/135 (16%) P<0.0001

Table 4. Table showing differences in FVIII, VWF(Ag), VWF activity and D-dimer in patients with normal and abnormal exercise test results.

Median levels (median (IQR))	Normal exercise test (n=220)	Abnormal exercise test (n=56)	
FVIII (NR 0.5-2IU/ml)	1.5 (1.2-2)	2 (1.6-2.4)	p<0.0001
VW Ag	1.3 (1.0-1.5)	1.5 (1.2-1.7)	P=0.0003

(NR 0.5-1.6IU/ml)			
VW activity (NR 0.5-1.87IU/ml)	1.2 (1-1.4)	1.2 (1-1.5)	P=0.3
D-dimer> 550 (NR 0-550ug/l FEU)	780 (633-1128)	780 (640-1315)	P=0.5

Table 5. VWF(Ag):ADAMTS13 ratios and exercise test results in 18 patients who had repeat assessments performed over time.

Patient	Assessment	VWF(Ag): ADAMTS13 ratio	Exercise test
1	Baseline 1 month	1.5 1.5	Lactate rise 0.8-2.4 Lactate rise and desaturation 3%
2	Baseline 1 month	1.7 1.5	Lactate rise 0.5-2.4 and desaturation 8% Desaturation 3%
3	Baseline 2 months	1.2 1.3	Not done Not done
4	Baseline 1 month	1.2 1.0	Normal exercise test Desaturation 3%
5	Baseline 5 months	1.3 1.4	Normal exercise test Not done
6	Baseline 4 months	0.6 0.7	Lactate rise 0.9-6.9 Not done
7	Baseline 1 month	1.2 1.0	Normal exercise test Normal exercise test
8	Baseline 1 month 4 months	1.3 1.5 1.3	Normal exercise test Desaturation 3% Not done
9	Baseline 1 month	1.7 1.7	Desaturation 13% and lactate rise 2.6-7.3 Desaturation 9% and lactate rise 0.5-8.7
10	Baseline 6 months	1.5 1.3	Normal exercise test Normal exercise test
11	Baseline 3 months	1.1 1.1	Normal exercise test Not done
12	Baseline	0.9	Normal exercise test

	1 month	0.9	Not done
13	Baseline	3.5	Normal exercise test
	3 months	3.6	Not done
14	Baseline	1.9	Normal exercise test
	1 month	0.7	Normal exercise test
	2 months	0.9	Not done
15	Baseline	2.0	Desaturation 3% and lactate rise 0.8 -2.4
	2 months	1.7	Normal exercise test
	3 months	1.6	Not done
	5 months	1.7	Not done
16	Baseline	1.5	Normal exercise test
	6 months	1.4	Normal exercise test
17	Baseline	0.5	Normal exercise test
	6 months	0.4	Not done
18	Baseline	2.8	Not done
	4 months	1.8	Not done

Figure 1a. Histogram showing distribution of the VWF(Ag):ADAMTS13 ratio across the post-COVID patient cohort.

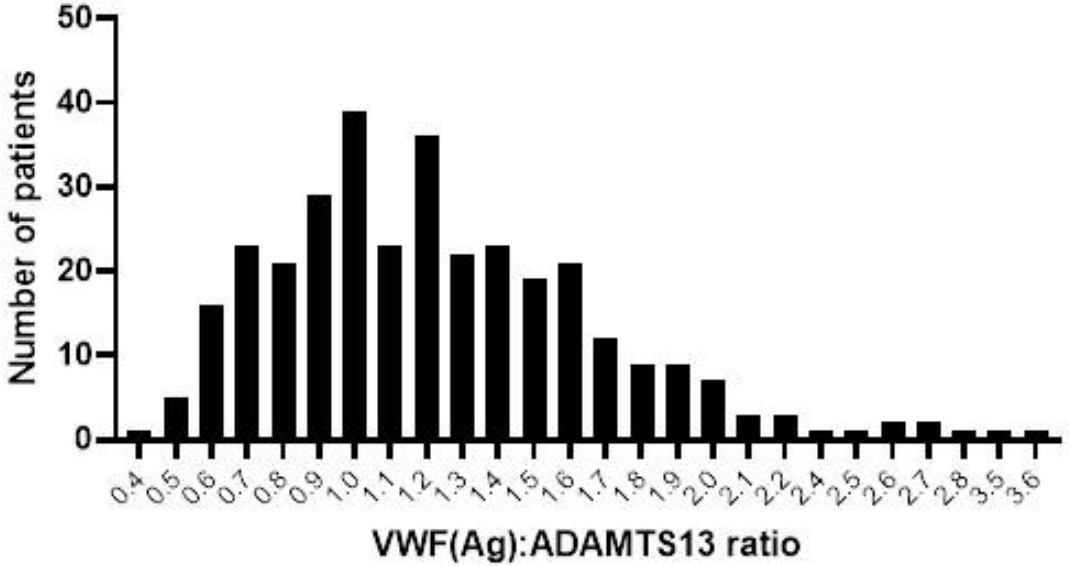

Figure 1b. Flowchart showing performance in exercise testing and associated VWF(Ag):ADAMTS13 ratio.

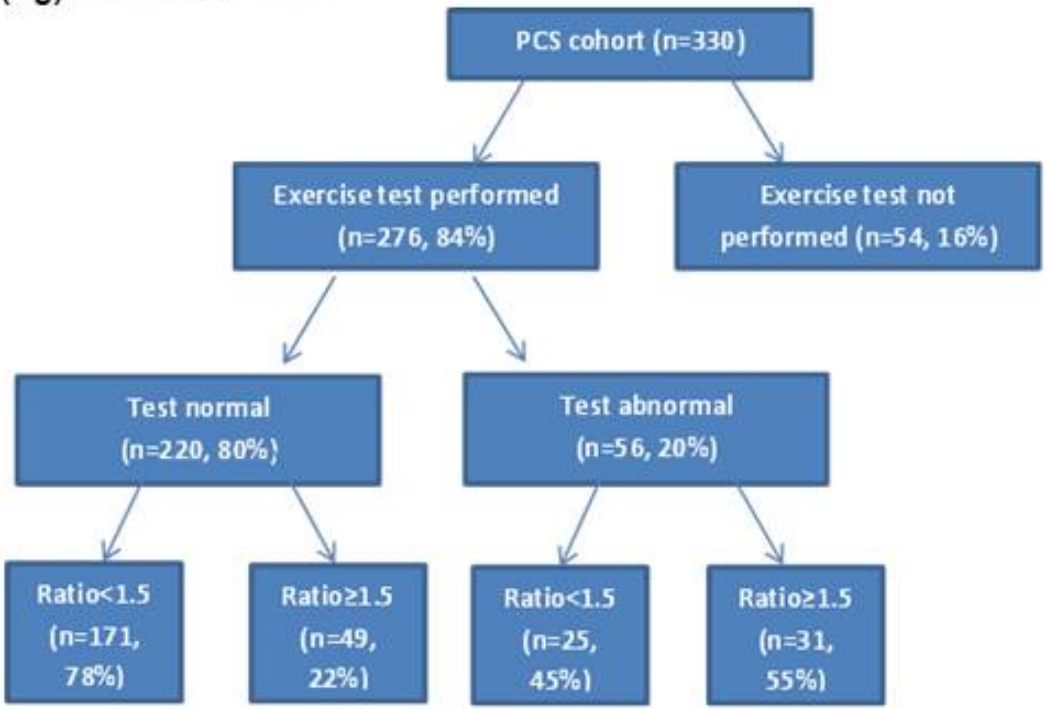

Figure 1c. Median VWF(Ag):ADAMTS13 ratio in patients with normal and abnormal exercise tests. Median VWF(Ag):ADAMTS13 ratio was 1.5 in patients with an abnormal exercise test compared to median ratio of 1.1 in patients with a normal exercise test (p<0.0001).